



Nitrogen uptake potential under different temperature-salinity conditions: Implications for nitrogen cycling under climate change scenarios

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ABSTRACT

As projected by climate change models, increase in sea surface temperature and precipitation in the future may alter nutrient cycling in the coastal regions due to potential changes in phytoplankton community structure and their ability to assimilate nitrogen (N) and carbon (C). An experiment simulating different temperature and salinity conditions (28°C–35 ambient conditions, 28°C–31, 31°C–35 and 31°C–31) in mesocosms containing 1000 L of coastal water from the Arabian Sea was performed and N uptake rates were measured using ¹⁵N tracer technique on 2nd, 5th, 7th and 10th day of the experiment. The results show that, under all conditions, the total N (NO₃[−] + NH₄⁺) uptake rates were lower in the beginning and on the final day of the tracer experiment, while it peaked during middle, consistent with chlorophyll *a* concentrations. Total N uptake rate was significantly lower (*p* = 0.003) under ambient temperature-lower salinity condition (28°C–31) than the others. This indicates that lowering of salinity in coastal regions due to excessive rainfall in the future may affect the N uptake potential of the phytoplankton, which may change the regional C and N budget.

1. Introduction

Observations of increase in global average air and ocean temperatures along with widespread melting of ice and rising sea level provide unequivocal evidence of climate warming (IPCC, 2007). Due to continued greenhouse gas emissions at or above present rates, climate models predict further warming in global climate system during 21st century. Several associated parameters of climate warming, such as increasing sea surface temperature (SST), ocean acidification, changes in vertical mixing, upwelling, precipitation and evaporation patterns, may in turn impact resilience of many ecosystems (Moore et al., 2008).

Increase in SST, one of the most important components of climate between oceans and atmosphere, is a major indicator of global climate warming. According to IPCC (2007) report, an average increase of ~1 °C in SST across the global ocean was evidently seen during the last century and is expected to increase further during the 21st century as well. An integrated study conducted from different platforms (satellite,

ships and buoys) has also shown upward trends in SST at all latitudes, with rates of change being greater than 2 °C per century in many tropical seas (Hoegh-Guldberg, 1999).

Salinity, another important characteristic of seawater, is one of the determining factors for water density, column stability and circulation patterns in the ocean. Unlike changes in SST, which follow relatively general global pattern, changes in sea surface salinity (SSS), particularly in coastal areas of the world, are largely determined by local meteorological processes, such as precipitation, evaporation and mixing (Henderson-Sellers and McGuffie, 2012). Precipitation data, in general, show considerable spatial and temporal variability. In the last century, precipitation has mostly increased over land, particularly in high northern latitudes and Asian regions, while decreases have dominated in tropics since the 1970s (IPCC, 2007). The frequency of extreme precipitation, however, has increased and different climate models agree with consistent increase in precipitation at higher latitudes and parts of tropics including Indian sub-continent for the 21st century

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(Bates et al., 2008).

Potential increase in SST and decrease in coastal SSS may be close to the physiological tolerance limits for different organisms (Walther et al., 2002). Marine phytoplankton, responsible for almost half of the global primary productivity (Field et al., 1998), may also get affected by such hydrographical changes, which can alter their nitrogen (N) and carbon (C) uptake potential. Phytoplankton species from both coastal (such as *P. tricornutum*) and open ocean waters (strains of *E. huxleyi* and *M. lutheri*) have shown intolerance towards change in temperature (Huertas et al., 2011). Studies have suggested that ocean warming has a significant impact on biological and ecological aspects, from species to community levels (Walther et al., 2002), including biodiversity, population size, species community composition, geographical distribution, phenology and productivity (Hardy, 2003; Edwards and Richardson, 2004; Behrenfeld et al., 2006; Jay and Marmot, 2009; Boyce et al., 2010; Moran et al., 2010). Models also predicted reduction in phytoplankton productivity due to increased SST during this century (Bopp et al., 2001; Steinacher et al., 2010; Thomas et al., 2012).

Within the context of climate change, few studies have explored temperature-related traits, such as optimal temperature for growth and thermal tolerance range for marine phytoplankton and other organisms (Kingsolver, 2009; Thomas et al., 2012). However, the response of the tropical phytoplankton community to the changing hydrographic conditions in terms of their production and N assimilation remains poorly understood. The extent of such hydrographic changes on the marine food web along with their potential to contribute directly or indirectly towards initiation of harmful algal blooms (HABs) and the proliferation of marine pathogenic bacteria also remains to be explored as the frequency, intensity, and geographical distribution of HABs appear to have increased over the last decades (Hallegraeff, 2010).

To explore the above-mentioned aspects, a mesocosm study simulating different temperature and salinity conditions was conducted on the natural pelagic bacterioplankton community from the coastal waters of the Arabian Sea, a tropical basin. The present work formed a part of this mesocosm study with the main objective to understand potential changes in N uptake capabilities of phytoplankton community under different temperature-salinity conditions. The simulated temperature-salinity conditions during the study attempted to mimic the range predicted by different climate models.

The Arabian Sea constitutes the north-western part of the Indian Ocean. Its uniqueness is the influence of seasonally reversing monsoonal wind forcings on the biogeochemical cycling, a characteristic which has attracted scientists worldwide to carry out biogeochemical studies. However, primary focus during major scientific programs such as Joint Global Ocean Flux Studies (JGOFS) or otherwise has been the open ocean with limited attention on coastal ecosystems (e.g., Madhupratap et al., 1996; McCarthy et al., 1999; Watts and Owens, 1999; Sambrotto, 2001; Kumar et al., 2010; Gandhi et al., 2010). Observations from the tropical Indian Ocean indicate positive SST anomaly concurrent with extreme monsoon rain events (Anderson et al., 2002; Goswami et al., 2006). Increased precipitation over central India leads to greater discharge of freshwater into the Bay of Bengal and the Arabian Sea, which create a negative SSS anomaly (Gopalakrishna et al., 2005; Nyadjro et al., 2012). In the coastal south-eastern Arabian Sea (SEAS), the SST displays a long-term oscillation pattern in concurrence with the El Niño Southern Oscillation (ENSO) with an amplitude of $> 4^{\circ}\text{C}$, and the SSS displays a negative linear trend of approximately 0.1 per year (Subrahmanyam et al., 2011; Godhe et al., 2015). Shift in phytoplankton community towards higher sample genus richness and diatom abundance have also been observed in the coastal regions of the SEAS (Parab et al., 2006). Given the importance of the Arabian Sea as a biogeochemical system and its potential to undergo changes due to warming climatic condition, it is prudent to examine the N uptake capabilities of its pelagic community under different temperature-salinity conditions, which is the aim of the present study.

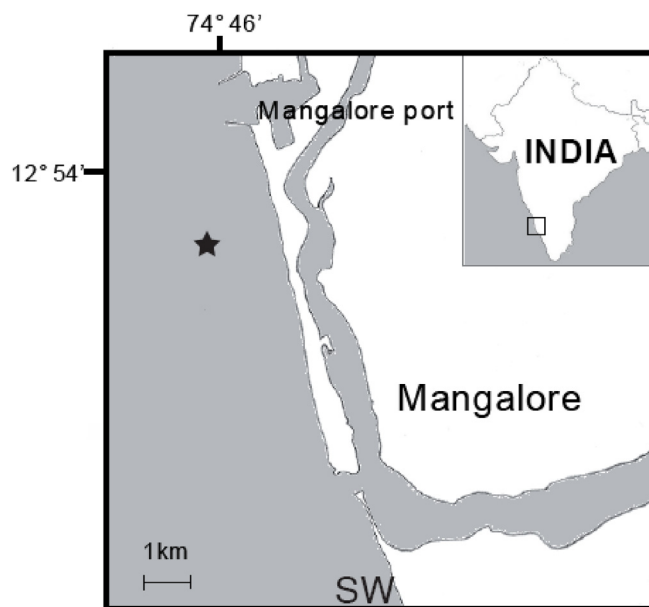


Fig. 1. Location of the sample collection for the mesocosm experiment. SW indicates the site for seawater collection. Star indicates the site for phytoplankton inoculum collection.

2. Materials and methods

2.1. Mesocosm setup

The present study was a part of a larger mesocosm study focussed on studying the response of coastal tropical pelagic bacterioplankton community to changing temperature and salinity. The biological aspects of the study, such as phytoplankton species composition, bacterial biomass, diversity index, and community changes during the course of the experiment, is discussed in a concurrent study (Kaur-Kahlon et al., 2016). We used these results appropriately in the present study.

For the whole study, sixteen mesocosms, each containing 1000 L of seawater, were used to simulate four different temperature-salinity conditions with four replicates for each condition. The conditions simulated were (1) ambient temperature - ambient salinity (28°C -35), (2) ambient temperature-decreased salinity (28°C -31), (3) increased temperature - ambient salinity (31°C -35), and (4) increased temperature-decreased salinity (31°C -31). Henceforth, for the purpose of discussion, these conditions will be referred as treatment 1 to 4, respectively.

The experiment was performed in Mangalore, west coast of India (Fig. 1). Seawater collected from the adjoining beach ($12^{\circ}47'024''\text{N}$, $74^{\circ}51'192''\text{E}$) during the first week of December 2011 was transported to the mesocosm facility and transferred in a common reservoir (20,000 L). Before the release of water into the mesocosms, the tanks were thoroughly scrubbed and wiped with ethanol. Seawater was filtered through $0.5\ \mu\text{m}$ sand filters and ozonated (Eltech ozone generator) to eliminate all living microorganisms, and simultaneously released into all the mesocosms.

The inoculum, phytoplankton assemblage with associated bacteria, was collected using plankton nets ($10\ \mu\text{m}$ mesh size) near the same location as the mesocosm water (Fig. 1). The method for concentrating the natural phytoplankton biomass is justified based on the dominance and functional importance of microphytoplankton in this and other coastal marine zones (e.g., McAllister et al., 1961). The phytoplankton species composition of coastal waters of the SEAS during the post-monsoon period is dominated by chain forming phytoplankton species (Asplund et al., 2011). When chain forming species, or species with spines or seate, occur in high abundances the plankton community itself forms a fine network inside the net which retains smaller solitary cells

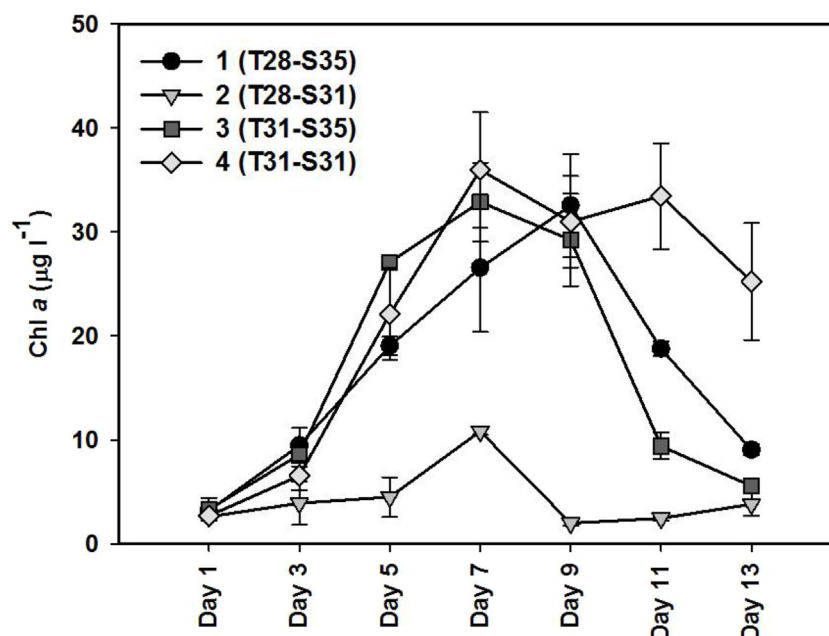


Fig. 2. Chlorophyll *a* concentrations (mean $\pm$ 1 σ) in the mesocosms. Numbers in the legend depict treatments 1 to 4 with respect to temperature and salinity.

(Tangen, 1978). Hence, the inoculum also represented cells smaller than the pore size of the net.

Inoculation was followed by a two-day period of gradual increase of temperature and decrease of salinity to avoid any sudden physical and metabolic shock to the inoculated microbial community. The manipulation was done step-wise by increasing the temperature and decreasing the salinity, respectively, every 6th hour. In treatment set up for increased temperature (31 °C), a set of 4 underwater heaters were placed to attain the same. Treatments with ambient temperature were also provided with a set of heaters to ensure a constant water temperature (28 °C). The lowering of salinity, from 35 to 31, was done by slowly extracting previously calculated volumes of seawater from the treatments through a submerged plankton net (10 μ m), not to lose or damage any cells, and thereafter adding equivalent volume of sterilized freshwater into the treatments. In the end, all mesocosms had the final volume of 1000 L and the equal biomass of phytoplankton cells (Kaur-Kahlon et al., 2016). Water temperature and salinity were identical among the four replicates within each treatment, initiating the subsequent sampling period.

In each treatment, surface light intensity was maintained at 50 μ mol photon $s^{-1} m^{-2}$ using four plant-growth fluorescent light lamps placed approximately 30 cm above water surface. The diurnal light cycle was set to 12 h. To maintain equal surface light conditions and to prevent unwanted particles from falling in, reflective blankets were put up above each tank with slits to facilitate air circulation. The treatments were mixed by continuous aeration, and gently stirred by a wooden stick (one for each mesocosm in order to avoid contamination among the different treatments) 30 min before sampling on alternative days. Nutrient concentrations (PO_4^{3-} , NO_3^- and NH_4^+) were measured spectrophotometrically (Grasshoff et al., 1999). The analytical precision for PO_4^{3-} , NO_3^- and NH_4^+ were ± 0.02 , ± 0.05 and $\pm 0.07 \mu M$, respectively.

2.2. Nitrogen uptake experiment

Four out of sixteen treatments, each belonging to different conditions, were selected for N uptake experiments. These experiments were performed on four different days (2nd, 5th, 7th and 10th day) of the whole experiment period, representing initial, peak and declining phase of phytoplankton growth. On each experiment day, water samples from

each treatment were collected into 1 L acid-washed polycarbonate Nalgene bottles in duplicate for NO_3^- and NH_4^+ uptake. Samples were spiked with 98 atom% enriched $Na^{15}NO_3$ and $^{15}NH_4Cl$ to trace NO_3^- and NH_4^+ uptake, respectively. Tracer additions were around 10% of the ambient concentrations. Subsequent to tracer additions, bottles were incubated inside respective mesocosm tanks for 4 h. Post-incubation samples were filtered onto precombusted GF/F filters (400 °C for 4 h) and subsequently oven dried (50 °C) for mass spectrometric analysis where ^{15}N atom% and particulate N (PN) content were measured using continuous flow stable isotope mass spectrometer (Delta V Plus, Thermo Scientific) connected to an elemental analyzer (Flash EA, 2000; Thermo Scientific). The coefficient of variation in ^{15}N atom% measurement was < 5% for NO_3^- and NH_4^+ uptake samples whereas the average difference in PN measurement of duplicate samples was < 8%. The uptake rate was calculated using the equation (Dugdale and Wilkerson, 1986; Kanda et al., 2003), Uptake rate ($\mu mol N l^{-1} h^{-1}$) = $P \times \Delta I_p / (t \times ((I_0 S_a + I_t S_t) / (S_a + S_t) - I_0))$, where: P is the amount of particulate N in post incubation sample; ΔI_p is the increase in ^{15}N atom% in particulate N during incubation; S_a and S_t are ambient and added NO_3^- (or NH_4^+) concentration, respectively; I_t and I_0 are ^{15}N atom% of added tracer and natural ^{15}N atom%; and t is the incubation time. This equation assumes no formation of nutrients during incubation and therefore rates presented here are potential rates.

Growth of phytoplankton biomass was monitored by spectrophotometrically measured photosynthetic active chlorophyll *a* (Chl*a*) concentrations. From each treatment, 200 ml of water sample in duplicate were vacuum-filtered through GF/F filters (Whatman). Chl*a* were extracted overnight at 4 °C, in 3 ml of 90% acetone. The absorption of the extracted pigments was measured in a spectrophotometer (Shimadzu, Japan). Chl*a* concentrations ($\mu g l^{-1}$) were calculated according to Jeffrey and Humphrey (1975).

2.3. Data analysis

Analysis of statistical significance was performed using ANOVA and *t*-test as a measure at $p < 0.05$. Factorial ANOVA to analyze the effects of temperature and salinity on uptake rates was also performed. Statistica Version 6.1 (Stat Soft. Inc.) was used to perform all the statistical analyses.

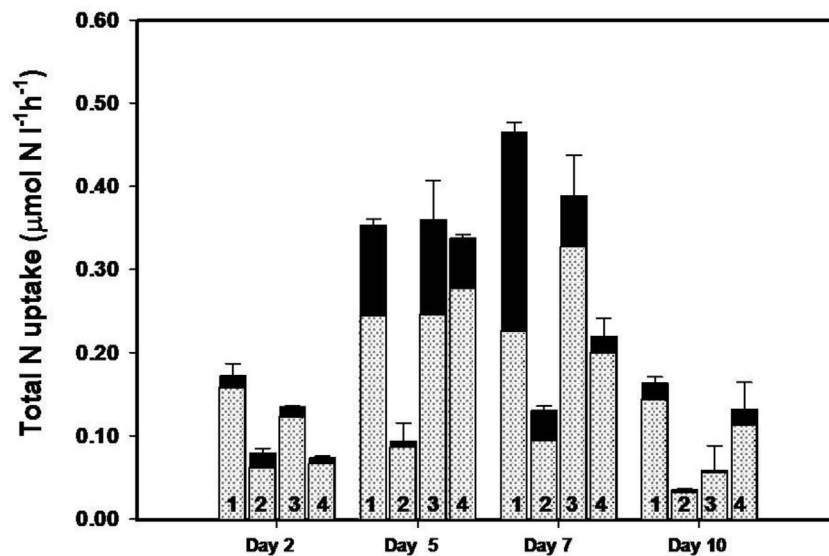


Fig. 3. Variations in N uptake rates in different treatments. Each bar symbolizes total N uptake rate (mean $\pm$ 1 σ). Dotted and black portion within each bar represent NH_4^+ and NO_3^- uptake rates, respectively. Numbers within bars depict treatments 1 to 4, respectively.

3. Results

Independent of treatment conditions, Chl *a* showed a consistent pattern with time. Chl *a* values were low during the initial phase of the experiment, peaked in the middle and reduced subsequently (Fig. 2). Chl *a* concentrations in all four mesocosms were less than $5 \mu\text{g l}^{-1}$ in the beginning, which increased to more than $30 \mu\text{g l}^{-1}$ during experimental day 7–11, except for treatment 2 (28°C -31). Overall, Chl *a* showed significant increase due to increase in temperature ($p = 0.01$) but under reduced salinity conditions its growth was minimum at ambient temperature.

For all treatments, consistent with Chl *a*, the total N uptake ($\text{NO}_3^- + \text{NH}_4^+$) rates also showed a similar pattern, i.e., relatively lower values during the beginning and the end of the experiment with peaks in the middle (Fig. 3). In general, the highest total N uptake rate on each experimental day was observed for treatment 1, maintained in ambient conditions (28°C -35), whereas treatment 2 with ambient temperature and reduced salinity (28°C -31) showed significantly lower values compared to the other treatments ($p = 0.003$). The highest total N uptake for treatment 2 was observed on the 7th day of the experiment ($0.13 \mu\text{mol N l}^{-1} \text{ h}^{-1}$), which was lower than the minimum ($0.16 \mu\text{mol N l}^{-1} \text{ h}^{-1}$) observed for treatment 1. In treatment 4, with increased temperature and reduced salinity (31°C -31), the total N uptake rates, in general, were higher than the treatment 2 (28°C -31). Between the two treatments at ambient salinity (35) where temperature was increased from 28 to 31°C (treatment 1 and 3), total N uptake rates did not differ significantly ($p = 0.47$, $\text{df} = 14$). However, total N uptake rates displayed an increase when temperature was increased while maintaining reduced salinity ($p = 0.01$, $\text{df} = 14$). Overall, total N uptake was significantly different among treatments ($p = 0.001$, $\text{df} = 14$) with significant decrease under reduced salinity ($p = 0.007$, $\text{df} = 30$).

Individually, NH_4^+ uptake rates followed similar pattern as total N uptake in which it increased over time to peak during experimental days 5–7, and decreased again at the end of the study period (Fig. 3). The lowest NH_4^+ uptake rate ($0.03 \mu\text{mol N l}^{-1} \text{ h}^{-1}$) was observed for treatment 2 (28°C -31) on the 10th day. NH_4^+ uptake rates in this treatment were significantly lower than that observed for other treatments ($p = 0.001$, $\text{df} = 30$). The highest NH_4^+ uptake ($0.32 \mu\text{mol N l}^{-1} \text{ h}^{-1}$) was observed for treatment 3 with increased temperature and ambient salinity (31°C -35) on experimental day 7. The NO_3^- uptake rates also followed similar trend as the NH_4^+ and the total uptake (Fig. 3). At the start of the experiment, the NO_3^- uptake rates were

similar for all treatments and increased on the 5th day except in treatment 2 (28°C -31), where it showed a decrease. The highest NO_3^- uptake ($0.23 \mu\text{mol N l}^{-1} \text{ h}^{-1}$) was observed in treatment 1 with ambient condition (28°C -35) on experiment day 7, when NO_3^- uptake rates for all other treatments were significantly lower. Overall, both NO_3^- ($p = 0.41$) and NH_4^+ ($p = 0.15$) uptake rates did not show significant change due to increase in temperature but decreased significantly at reduced salinity ($p = 0.01$ and $\text{df} = 30$ for both). NO_3^- and NH_4^+ uptake rates during the present study were in the range observed for the coastal Arabian Sea (NO_3^- uptake rates: 0.003 – $0.18 \mu\text{mol N l}^{-1} \text{ h}^{-1}$ and NH_4^+ uptake rates: 0.02 – $0.34 \mu\text{mol N l}^{-1} \text{ h}^{-1}$; Bhavya et al., 2016).

Ammonium was the preferred substrate for phytoplankton under all treatments (Fig. 4). In the beginning of the experiment, NO_3^- uptake contribution to the total uptake was less than 10% for all treatments, except in treatment 2 (28°C -31), where it was 20%. However, the contribution of NO_3^- uptake increased during experiment days 5–7, but reduced again towards the end. Overall, NO_3^- uptake was less than 32% of the total uptake, except for treatment 1 (28°C -35) on experiment day 7 in which it was approximately 50%.

For all treatments, calculations for total N uptake per unit Chl *a* showed an interesting pattern with gradual decline over time (Fig. 5). High total N uptake per unit Chl *a* ($\mu\text{g N} (\mu\text{g Chl } a)^{-1} \text{ h}^{-1}$) were observed at the start of the experiment with the highest value (0.35) for treatment 1 (28°C -35). Interestingly, contrary to general trend observed for other treatments, this value showed slight increase from 7th (0.17) to 10th (0.20) day for treatment 2, the only such increase during this study.

Results of categorical ANOVA suggested salinity to be a significant factor (total N uptake: $p = 0.0005$, $F = 8.94$; NO_3^- uptake: $p = 0.01$, $F = 6.26$; NH_4^+ uptake: $p = 0.01$, $F = 7.35$) affecting N uptake rates during the present study. The effect of temperature (total N uptake: $p = 0.52$, $F = 0.41$; NO_3^- uptake: $p = 0.36$, $F = 0.84$; NH_4^+ uptake: $p = 0.10$, $F = 2.73$) was relatively insignificant.

In all the treatments, NH_4^+ uptake rates did not show significant relationship with substrate concentrations ($R^2 = 0.002$, $p = 0.78$; Fig. 6a). The NO_3^- uptake rates showed a weak negative relationship ($R^2 = 0.14$, $p = 0.06$) which was mainly driven by high uptake rate in treatment 1 on day 7, without which the relationship became insignificant ($R^2 = 0.01$, $p = 0.48$; Fig. 6b). The average initial NO_3^- ($8.4 \pm 1 \mu\text{M}$) and NH_4^+ ($2.0 \pm 0.5 \mu\text{M}$) concentrations during the experiment were high with relatively higher concentrations in

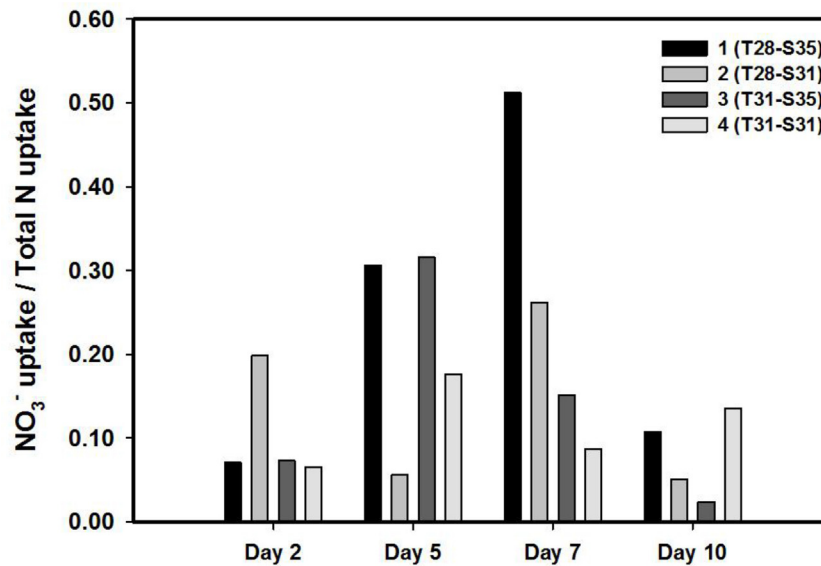


Fig. 4. Ratio of NO_3^- uptake rates to total N ($\text{NH}_4^+ + \text{NO}_3^-$) uptake rates in different treatments. Numbers in the legend depict treatments 1 to 4 with respect to temperature and salinity.

treatment 4 compared to others (Fig. 7), possibly due to regeneration of nutrients because of initial increased temperature. The minimum NO_3^- and NH_4^+ concentrations were around 1.0 and 0.6 μM , respectively, except for the last day of experiment for treatment 1 ($\text{NO}_3^- \sim 0.22 \mu\text{M}$). The pattern of change in NH_4^+ concentration in treatment 2 during the course of experiment was different than the other treatments (Fig. 7). The build-up of NH_4^+ on day 7 in this treatment coincided with the higher NO_3^- uptake. PO_4^{3-} concentrations averaged around 1 μM with N:P ratios maintained at 7–22 throughout the mesocosms experiment.

4. Discussion

Understanding the response of phytoplankton in tropical ecosystems, such as coastal Arabian Sea, due to ocean warming and changing salinity is vital, if we are to fully appreciate the potential alterations in N and C cycle due to climate change. Results from the present mesocosm study indicate the importance of salinity and temperature in

modulating potential N uptake by the coastal phytoplankton community. Nitrogen uptake rates showed significant decline due to the lowering of salinity whereas, increase in temperature appears to augment it at reduced salinity. This dependence of N uptake on temperature and salinity in nutrient replete environments, such as coastal zones, may have important consequences for N cycling in tropical ecosystems in future due to climate change.

The findings of this study become important as climate change due to global warming has been predicted to be the largest anthropogenic disturbance on the natural ecosystems in the 21st century (Thomas et al., 2004; Deutsch et al., 2008). However, the impact of climate change on phytoplankton will depend on both the magnitude and pattern of climate change along with the thermal sensitivity of the species involved. A recent study suggests that optimum temperature for phytoplankton growth in temperate and polar regions is considerably higher than their mean annual temperature, whereas it is closer to mean temperature in the tropical region (Thomas et al., 2012). Because of this thermal dependency, it has also been predicted that the

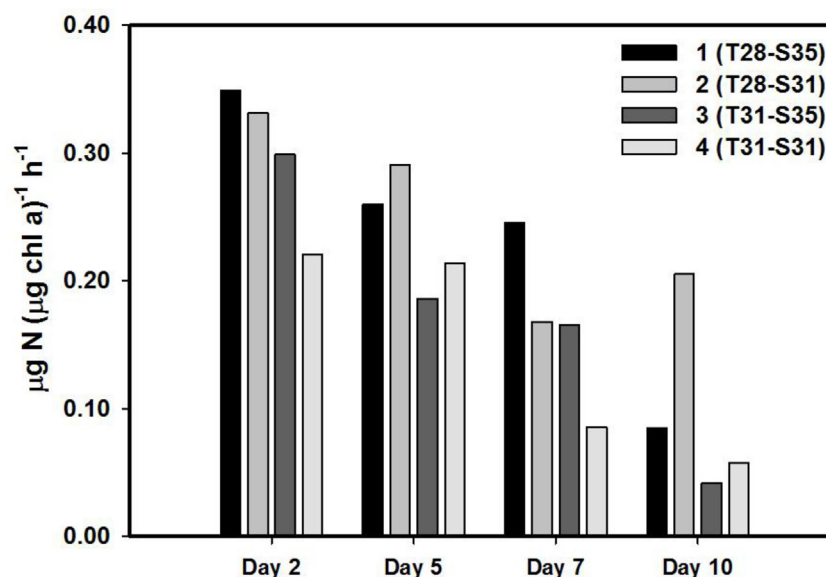


Fig. 5. Total N uptake per unit Chl a in different treatments. Numbers in the legend depict treatments 1 to 4 with respect to temperature and salinity.

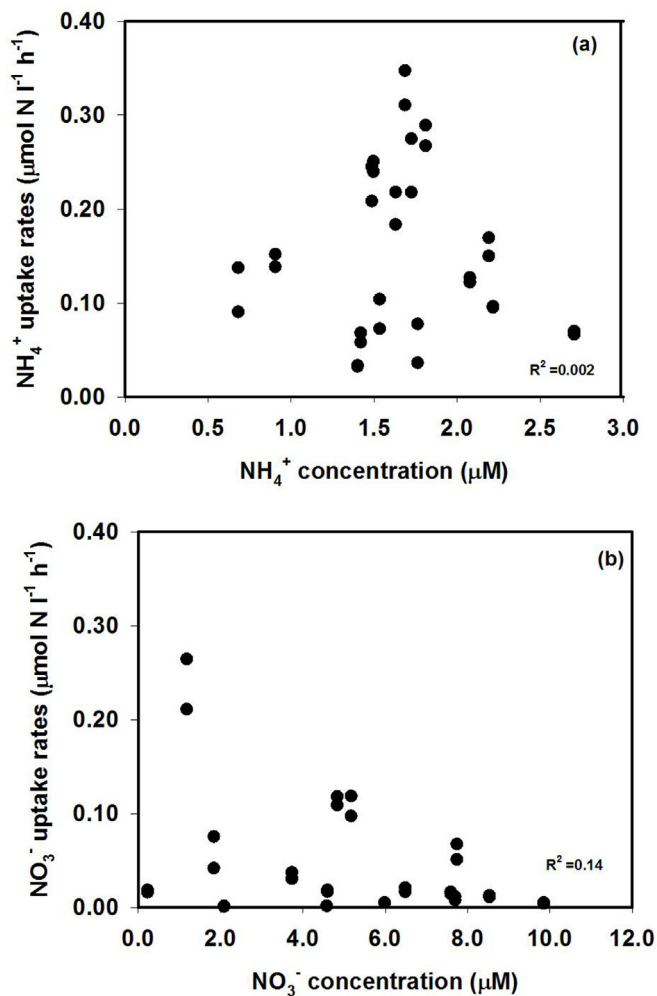


Fig. 6. Relationship between (a) NH_4^+ concentrations and NH_4^+ uptake rates, and (b) NO_3^- concentrations and NO_3^- uptake rates.

phytoplankton community in tropical waters are more susceptible to climate change than the ones in temperate and polar regions; and an equivalent increase in temperature can lead to faster decline in growth rate of tropical phytoplankton (Hooper et al., 2012; Thomas et al., 2012). Overall, the role of substrate availability, temperature, and salinity on biota from different climatic zones needs to be thoroughly explored.

Our parallel studies by Kaur-Kahlon et al. (2016) have presented the

details on biological aspects of this mesocosm experiment. Briefly, these findings revealed that the phytoplankton inoculum collected from the field was almost exclusively made up of diatoms and this group dominated the phytoplankton community in all the treatments. Towards the later phase of the experiment, smaller ($< 5 \mu\text{m}$) flagellates and heterotrophic dinoflagellates *Protoperdinium* spp. increased proportionally in the decreased salinity manipulated conditions. *Skeletonema* sp. was dominant in all the treatments probably due to its inherent plasticity. The community changes showed inverse relationship between species richness and total phytoplankton abundance in all mesocosms irrespective of treatment. Increased temperature displayed a significant decline of diversity index over time caused by the proliferation of the fast growing and opportunistic species. In contrast, decreased salinity counteracted the fast decay in species diversity by allowing estuarine species to prosper to a greater extent, which is in agreement with the studies of Härnström et al. (2007) in the Mangalore coastal region. Phytoplankton biomass had a strong direct positive influence on the bacterial biomass; the latter was not only directly affected by the treatment (temperature and salinity changes) but also indirectly affected through phytoplankton biomass and its response to the treatment.

The observed variations in N uptake rates during the present study appear to be largely due to the modulating role played by simulated temperature and salinity conditions. Generally, the mechanism of nutrient supply and its kinetics of utilization determine the productivity and size structure of phytoplankton (Harrison et al., 1996), which follows concentration-dependent uptake of dissolved inorganic N according to the Michaelis-Menten kinetics for enzymes (Menten and Michaelis, 1913). But it appears that under nutrient replete tropical conditions, factors like temperature and salinity become important. It has been reported earlier that kinetic relationships, such as nutrient uptake, exhibit wide range of variability even for a given substrate and species. These relationships can be a function of physiological state of the phytoplankton cells, time of exposure to nutrients and environmental factors (Glibert et al., 2013). Nevertheless, the lack of significant relationships between nutrients concentration and respective uptake rates during the present study (Fig. 6) favours to argue that under changing hydrographical conditions the N uptake potential of coastal tropical phytoplankton may tend to behave independent of ambient substrate concentrations. The N:P ratios (7–22), which were sufficiently maintained throughout the experiment in all the treatments, further supports that phytoplankton growth and their uptake are not limited by nutrients availability.

The significantly lower total N uptake rates ($p = 0.001$, $df = 14$) for the treatment with reduced salinity and ambient temperature entail important consequences for potential N uptake in the coastal Arabian Sea under increased precipitation. But total N uptake rates displayed an increase when temperature was increased while maintaining reduced

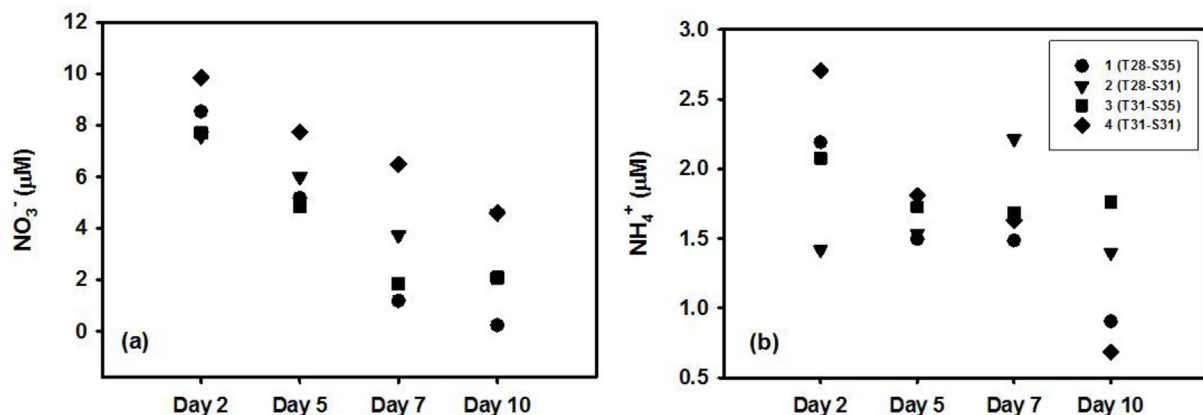


Fig. 7. Variations in NO_3^- and NH_4^+ concentrations in different treatments during the course of experiment.

salinity ($p = 0.01$, $df = 14$). This implies that elevated temperature probably favours increase in N uptake at reduced salinity. However, treatment with ambient conditions shows higher N uptake, which probably is due to adaptation and affinity to local environmental conditions. Overall, N uptake potential in tropical coastal ecosystem, such as during the present study, may be an interplay between salinity and temperature conditions. While N uptake rates tend to decrease with the reduction in salinity, slight increase in temperature at reduced salinity may contribute to its increase. Thomas et al. (2012) reported a wide range (approximately 22–32 °C) of optimum temperature for the growth of phytoplankton strains from the tropical geographical region where the inoculum was collected for the present experimental study (Arabian Sea). As the tropical SSTs are very close to this maximum value, they have predicted that further increase in temperature may be adverse for growth of phytoplankton in this region. In such case, the thermal niche width, the temperature range over which growth rate remains positive is highly critical.

Few studies exist to show the effect of salinity and temperature stress on N uptake rates. A limited number of studies from estuarine ecosystem have observed relationship between uptake rates and salinity (e.g., Middelburg and Nieuwenhuize, 2000; Mulholland et al., 2003). In a different salinity range (0.03–10.5), higher N uptake has been observed in higher salinity waters in a sub-estuary of the Chesapeake Bay despite lower NO_3^- and NH_4^+ concentrations compared to freshwaters (Mulholland et al., 2003). Similar trends have been observed in the Cochin estuary, a tropical ecosystem on the southwest coast of India, during dry season (Bhavya et al., 2016). This appears to show that salinity has a role to play in modulating N uptake rates.

Treatment 1 with ambient conditions has showed responses typical of a diatom dominated community but manipulated salinity conditions have showed maximal variations as evidenced by other treatments. There is a possibility that reducing salinity from 35 to 31 in 48 h in definite intervals (6 h) causes osmotic stress related changes in the N uptake. A two-point reduction in salinity in 24 h, which is near the doubling time for diatoms of tropical waters (Suman et al., 2012; Chari et al., 2013), may also compromise the growth and nutrient uptake of phytoplankton. Possibility of increase in cell lysis due to lowering of salinity and thereby reducing phytoplankton biomass and N uptake also exists. However, under such circumstances, dissolved organic carbon (DOC) concentration is expected to be higher (Passow et al., 2007) particularly in the low salinity treatment. In contrast, relatively low and uniform DOC concentrations among the treatments shows no signs of osmotic stress or cell lysis during the course of the experiment (Kaur-Kahlon et al., 2016).

Independent of mesocosm conditions, NH_4^+ was assimilated in preference to NO_3^- and primary production appeared to be mainly supported by NH_4^+ based regenerated N. Relative preference index (RPI) calculated based on McCarthy et al. (1977) showed values much less than 1 for NO_3^- , which indicates preference for NH_4^+ . The preference for reduced or recycled forms of N such as NH_4^+ is well known in aquatic environments (e.g., Dugdale and Goering, 1967; McCarthy and Eppley, 1972; Glibert et al., 1982) due to higher energy requirement for the assimilation of NO_3^- (McCarthy and Carpenter, 1983).

Although contribution of NO_3^- to the total uptake increased during the middle phase of the experiment compared to the beginning and the end, it remained lower than the NH_4^+ uptake throughout, except in the ambient treatment during bloom phase (day 7). It appeared that some physical or biological controls were limiting NO_3^- utilization despite high primary productivity, which has been observed in the open Arabian Sea (McCarthy et al., 1999) as well as in the Mangalore coastal waters (Bhavya et al., 2016). Inhibition of NO_3^- uptake in the presence of NH_4^+ due to adverse effect on NO_3^- metabolism could be one of the reasons for lower NO_3^- uptake, a commonly observed feature in the world oceans (e.g., Glibert et al., 1982; Harrison et al., 1987; McCarthy et al., 1999). Although the extent of inhibition is much more variable, the inhibition is potentially greater under nutrient replete conditions

and the least when phytoplankton are N-starved (Conway, 1977; Dortch, 1990). During the present study, ambient conditions were nutrient replete and this might have contributed to relatively lower NO_3^- uptake. Yet another reason could be higher than required addition of $^{15}\text{NH}_4^+$ tracer during the experiment, which could trigger artificial stimulation of NH_4^+ uptake (Watts and Owens, 1999). However, the addition of NH_4^+ in our experiments was not large enough to stimulate an artificial uptake, which was evident by very weak correlation ($r^2 \sim 0.006$) between the percentages of NH_4^+ addition relative to ambient and NH_4^+ uptake rates for all mesocosms. One more reason for NO_3^- underutilization could be iron and silicon limitation (Landry et al., 1997), but the concentrations of iron (Measures and Vink, 1999) and silica (Morrison et al., 1998) in the Arabian Sea, especially in the coastal seas (for silica see Gupta et al., 2016), are greater than the known limiting values.

Phytoplankton, especially during bloom period (day 7), showed higher affinity towards NO_3^- at ambient temperature and towards NH_4^+ at increased temperature at both salinity levels (Figs. 3 and 4). Contrary to changes with reduced salinity, no temperature related changes were evident in total N uptake under ambient salinity. Interaction of increased temperature and reduced salinity appear to stimulate higher NH_4^+ uptake (Fig. 3). Increase in NO_3^- uptake was seen in reduced salinity under ambient but not in the increased temperature treatment between pre-bloom and bloom conditions. This infers that salinity related changes appear to affect both NO_3^- ($p = 0.01$) and NH_4^+ uptake ($p = 0.01$), whereas temperature related ones mildly affect only NH_4^+ uptake ($p = 0.10$). Experiments with larger sample size are required to unequivocally establish this trend. Changes in species composition in reduced salinity treatments from large diatoms like *Coscinodiscus* sp. and *Heliotheca tamensis* during the early days of experiment to small pennate diatoms (*Thalassiosira* sp.) and autotrophic nanoflagellates during the blooming phase (Kaur-Kahlon et al., 2016) might have supported relatively higher NO_3^- uptake. However, this remains to be corroborated due to lack of studies related to size fractionated NO_3^- uptake by diatoms in the SEAS. A positive correlation of diatoms species diversity with temperature, salinity and nutrients in the SEAS (Rai and Rajashekhar, 2014) infers that diatoms diversity is higher in the dry conditions and lower with lowering of salinity. Importantly, microphytoplankton blooms and their species composition (dominated by chain forming diatoms) in the SEAS during summer monsoon are emanated from the combined effects of reduction in salinity due to precipitation and NO_3^- enrichment from coastal upwelling (Lathika et al., 2013; Gupta et al., 2016). It is prudent to have details on < 20 μm size fraction and their dynamics also in the SEAS to compare our experimental results but unfortunately this information is not available. But it is likely that under the nutrients replete conditions the preferential uptake of NO_3^- and NH_4^+ is more pronounced by the temperature and salinity variations in the natural systems, which in turn affect the phytoplankton size variations and diversity.

The present study did not focus on the specific importance of phytoplankton size and heterotrophic bacteria on N uptake rates. Therefore, it is not possible to decouple the contribution of bacteria to the variabilities observed in NO_3^- and NH_4^+ uptake rates due to changing temperature and salinity. Smaller size phytoplankton and heterotrophic bacteria are known to play a significant role in N uptake (Laws et al., 1985; Kirchman, 1994; Gardner et al., 2004). Since climate change related models predict a change in auto-heterotrophic community size structure, the utilization of different N sources could be altered due to high preference for NH_4^+ by smaller size phytoplankton as well as autotrophic and heterotrophic bacteria. Size-specific prey-predator relationships could also lead to a change in NO_3^- and NH_4^+ consumption. Hence, the individual response of bacteria and picoplankton under such simulated conditions as during the present study warrants attention in future. Also, the extent of dissolved organic N as an alternative source of N was not considered in this study and could be a component of N uptake in future studies.

5. Conclusions

Our results suggest that N uptake potential of the coastal tropical ecosystems such as the coastal Arabian Sea may decrease with lowering of salinity. This may have important consequences for N budget of tropical coastal ecosystems, if there is an increase in seasonal precipitation or extreme precipitation events in future due to climate change. This study also underscores the importance of temperature and salinity on N uptake potential under nutrient replete conditions, virtually making N uptake a function of these two parameters. It is important to consider that the studied changes take time in nature and fast-growing species have shown capabilities of adaptation to climate change conditions, hence the predictability of future changes vis-à-vis our findings needs cautious approach.

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